**This is from a previous year so the questions may not all be the exact same, but I think it’s sufficient**

**BACTERIAL TOXINS**

**Botulinum and tetanus exotoxins. (AB toxins, Zinc proteinase activity)**

**3.** Distinguish the clinical signs for the types of paralysis caused by tetanospasmin and botulinum toxin.(Medline Plus Dictionary, Dynamed, Medical Microbiology 4th ed. Samuel Baron)

a. Tetanospasmin causes muscle twitching and cramping that is initially located around a wound. Some parts of the body may become extremely rigid. Opisthotonos is common. (def.- a spasm in which the head and heels are bent backward and the body bowed forward).

i. Spastic Paralysis: paralysis with tonic spasm of the affected muscles and with increased tendon reflexes

b. Botulinum toxin causes a characteristic, symmetrical, descending paralysis. Multiple cranial nerve palsies and descending flaccid paralysis.

Flaccid Paralysis: paralysis in which muscle tone is lacking in the affected muscles and in which tendon reflexes are decreased or absent

**Superantigens**

**1.** Explain the mode of action of superantigens (*e.g.* TSST-1, staphylococcal enterotoxin) and the typical clinical signs/symptoms. (DynaMed, Access Medicine, Endotoxin and Septic Shock lecture)

a. Superantigens bind to MHC class II of antigen presenting cells on the outside of the binding groove. By binding to the outside of the MHC II, it causes it to be recognized by the Vβ element of TCR. Therefore any T cell with the Vβ element can be stimulated, bypassing antigen specificity.Superantigens can activate up to 20% of peripheral T-cells.

b. Activated T-cells release cytokines. Symptoms include extremely high fever (>102), hypotension (due to increased vascular permeability), and extreme dehydration.

**2.**  Explain why not all T-Cells become activated by any one superantigen.

a. Not all T-cells have the required Vβ element that is necessary for activation by a superantigen.

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**Bacterial Toxins**:

**Describe the typical stages of exotoxin interaction with a target, including:**

- Binding to a receptor

- Being internalized

- Being transported to target (*e.g.*, via endosomes, lysosome, lipid rafts, *etc.*)

- Acting on specific target

There are at least **two mechanisms of toxin entry into target cells.**

In one mechanism called **direct entry**, the B subunit of the native (A+B) toxin binds to a specific receptor on the target cell and induces the formation of a pore in the membrane through which the A subunit is transferred into the cell cytoplasm.

In an alternative mechanism, the native toxin binds to the target cell and the A+B structure is taken into the cell by the process of **receptor-mediated endocytosis** (**RME**). The toxin is internalized in the cell in a membrane-enclosed vesicle called an **endosome**. H+ ions enter the endosome lowering the internal pH which causes the A+B subunits to separate. The B subunit affects the release of the A subunit from the endosome so that it will reach its target in the cell cytoplasm. The B subunit remains in the endosome and is recycled to the cell surface.

In both cases above, a large protein molecule must insert into and cross a membrane lipid bilayer, either the cell membrane or the endosome membrane. This activity is reflected in the ability of most A+B or A/B toxins, or their B components, to insert into artificial lipid bilayers, creating ion permeable pathways. If the B subunit contains a hydrophobic region (of amino acids) that insert into the membrane (as in the case of the diphtheria toxin), it may be referred to as theT (translocation) domain of the toxin.

As proteins, many bacterial toxins **resemble enzymes** in a number of ways. Like enzymes, they are **denatured by heat**, acid and proteolytic enzymes, they **act catalytically**, and they exhibit **specificity of action**. The **substrate** (in the host) may be a component of tissue cells, organs or body fluid.

Some protein toxins have very **specific cytotoxic activity** (i.e., they attack specific types of cells). For example, tetanus and botulinum toxins attack only neurons. But some toxins (as produced by staphylococci, streptococci, clostridia, etc.) have fairly **broad cytotoxic activity** and cause nonspecific death of various types of cells or damage to tissues, eventually resulting in necrosis. Toxins that are phospholipases act in this way. This is also true of pore-forming hemolysins and leukocidins.

**Explain that exotoxins often are key virulence factors and, in many cases, toxoid forms are used as vaccines.**

Exotoxins are key virulence factors! The net effect of the toxin depends on the function of the target protein and the function of the cell. If it is crucial for the protein-synthesizing apparatus of the cell (like diphtheria toxin), protein synthesis ceases and **the cell dies**. However, cell death is not the inevitable outcome of toxin action. One of the major targets of the ADP-ribosylating A–B toxins are guanine nucleotide-binding proteins (G proteins), which are involved in signal transduction in eukaryotic cells. In this case, the inactivation of the G protein can inhibit or stimulate some activity of the cell. Examples: cholera toxin inactivates a G protein that down-regulates a secretory pathway. If the cell is an intestinal enterocyte, the end result is hypersecretion of electrolytes and diarrhea. Cholera toxin applied to cells from the adrenal gland stimulates steroid production.

(Ryan KJ, Ray CG, "Chapter 22. Pathogenesis of Bacterial Infections" (Chapter). Ryan KJ, Ray CG: Sherris Medical Microbiology, 5e:<http://0-www.accessmedicine.com.carlson.utoledo.edu/content.aspx?aID=6941709>.)

The following bacteria have vaccines with their exotoxin: *Corynebacterium diphtheriae, Clostridium tetani, Bordetella pertussis* (Diphtheria, tetanus, and pertussis).

**Diphtheria**:

**1. Describe the mode of action of diphtheria toxin including its binding to target cells and its action on the cellular target.**

Diphtheria toxin is the major virulence factor of *C. diphtheriae*. This exotoxin is produced at the site of infection and then disseminates through the blood to produce the systemic signs of diphtheria. The organism doesn’t need to enter the blood to produce disease. The toxin protein is an example of the classic A-B exotoxin. 3 functional regions exist on the toxin molecule, a **receptor-binding region** and a **translocation region** on the B subunit and a **catalytic region** on the A subunit. The receptor for the toxin is **heparin-binding epidermal growth factor**, which is present on the surface of many eukaryotic cells, particularly heart and nerve cells; its presence explains the cardiac and neurologic symptoms observed in patients with severe diphtheria. After the toxin becomes attached to the host cell, the translocation region is inserted into the endosomal membrane, facilitating the movement of the catalytic region into the cell cytosol. The A subunit then terminates host cell protein synthesis by inactivating **elongation factor 2 (EF-2)**, a factor required for the movement of nascent peptide chains on ribosomes. Because the turnover of EF-2 is very slow, and approximately only one molecule per ribosome is present in a cell, it has been estimated that one exotoxin molecule can inactivate the entire EF-2 content in a cell, completely terminating host cell protein synthesis. Toxin synthesis is regulated by a chromosomally encoded element, **diphtheria toxin repressor (DTxR)**. This protein, activated in the presence of high iron concentrations, can bind to the toxin gene operator and prevent toxin production. Murray, pg 261-262.

***2.* Explain the association of diphtheria toxin production with the presence of latent bacteriophage in *Corynebacterium diphtheriae.***

The *tox* gene that codes for the endotoxin is introduced into strains of *C. diphtheriae* by a lysogenic bacteriophage (**β-phage**). Two processing steps are necessary for the active gene product to be secreted: (1) proteolytic cleavage of the leader sequence from the toxin protein during secretion from the bacterial cell; and (2) cleavage of the toxin molecule into two peptides (A and B) that remain attached by a disulfide bond. Murray, pg 261.

**3. Describe the nature of the vaccine used to prevent diphtheria.**

The vaccine contains the toxoid (formaldehyde-treated exotoxin). Immunization against diphtheria is indicated for every child. Route is intramuscular.

Vaccine is often combined with tetanus and pertussis in a vaccine known as DTaP or DTP or Tdap. DTaP (alone or combined with other vaccines) is used for infants and children between 6 weeks and 6 years of age. There is no pertussis-containing vaccine licensed for children aged 7–9 years, so Td is used for this age group when tetanus and diphtheria vaccination is needed. **For adolescents and adults, a single dose of Tdap is used, followed by booster doses of Td every 10 years**.

The **primary** series of DTaP vaccination should consist of **four doses**, given at 2, 4, 6, and 15–18 months of age. The fourth dose may be given as early as 12 months of age if 6 months have elapsed since the third dose. Giving the fourth dose between 12 and 15 months of age is indicated if the provider thinks the child is unlikely to return for a clinic visit between 15 and 18 months of age. Children should receive a fifth dose of DTaP at 4–6 years of age. However, a fifth dose of DTaP is not needed if the fourth dose was given after the child's fourth birthday. The same brand of DTaP should be used for all doses if feasible. (Various AccessMedicine sources combined into a beautiful answer).

**4. Explain the treatment of clinical diptheria**

Antitoxin, which is prepared from horse serum, must be given in all cases when diphtheria is suspected. For mild early pharyngeal or laryngeal disease, the dose is 20,000–40,000 units; for moderate nasopharyngeal disease, 40,000–60,000 units; for severe, extensive, or late (3 days or more) disease, 80,000–100,000 units. Diphtheria equine antitoxin can be obtained from the CDC. Removal of membrane by direct laryngoscopy or bronchoscopy may be necessary to prevent or alleviate airway obstruction.

Either penicillin, 250 mg orally four times daily, or erythromycin, 500 mg orally four times daily, for 14 days is effective therapy, although erythromycin is slightly more effective in eliminating the carrier state. Azithromycin or clarithromycin is probably as effective as erythromycin. The patient should be isolated until three consecutive cultures at the completion of therapy have documented elimination of the organism from the oropharynx. Contacts to a case should receive erythromycin, 500 mg orally four times daily for 7 days, to eradicate carriage.

**5. Describe the persons in the USA who are most susceptible to developing diptheria**

Due to the Tdap vaccine, there have only been 3 reported cases between 2000 and 2007. The most likely person to in the USA to develop diphtheria are those who have not had an updated vaccine and are travelling to countries where the disease is more prevalent.

**FOOD SAFETY**

**1. What are the main causes of gastroenteritis and food poisoning in the USA? Note that viruses are a more common cause of gastroenteritis than bacteria, but much of this may be fecal-oral rather than directly caused by food poisoning.**

Norovirus (Norwalk virus) causes 50-70% of gastroenteritis cases. Rotovirus is the leading cause of diarrhea in kids <5 yo, but a vaccine now exists and is used in the US, so this is not the case within the US. S. aureus is a common bacterial cause of food poisoning. The majority of food-borne illnesses are due to viruses, however.

**2. Which foods are dangerous is not handled correctly? Make sure you include common sources of Campylobacter, Salmonella, E. coli, Listeria, and viruses, and identify some of the symptoms and signs associated with poisoning by these agents.**

Pretty much all food can be dangerous if not handled correctly, particular meat and meat products, poultry and eggs, and dairy.

Norovirus (most common cause of GE) and other viruses can contaminate anything that has been handled by someone who has been infected. Viruses are fecal-oral spread in most cases, so proper hygiene is of utmost importance.

signs and symptoms: abrupt onset of vomiting, diarrhea, cramps, and nausea usually

24-48 hrs after consumption

Campylobacter- found in raw/undercooked poultry, unpasteurized milk, contaminated water, and anything affected by cross-contamination

signs and symptoms: diarrhea, cramping, fever w/in 2-5 d after consumption, may have

bloody diarrhea with nausea and vomiting

Salmonella- foods contaminated w/ animal feces (beef, poultry, milk, eggs, vegetables), can get

from turtles

signs and symptoms: diarrhea, cramping, fever 12-72 hrs after consumption

E. coli- raw meat, milk and dairy, raw fruits/vegetables if they have come in contact with feces

signs and symptoms: watery or bloody diarrhea, cramps, with or w/o fever 1 d-1 wk after

consumption

Listeria- meat and dairy (unpasteurized), raw vegetables, processed meats, smoked seafood

signs and symptoms: diarrhea and other GI symptoms w/ fever and muscle aches (no

incubation period given)

**3. What infectious disease is most commonly transmitted via eggs? Do you expect regulations concerning the cooking of eggs, as well as the intro of pasteurized eggs and egg product to affect the incidence of disease?**

Salmonella

Current regulations/suggestions: store in fridge <40 F, don’t use cracked or dirty eggs, wash after handling raw egg and disinfect surfaces, cook thoroughly (to 160 F, internal temp), eat promptly after cooking (within 2 hrs), buy pasteurized

It seems like following these suggestions would decrease incidence of disease. However, I don’t think anyone could convince an old man not to have his eggs from Bob Evans sunny-side-up. :)

**4. Bacterial contamination can lead to production of toxins by the bacteria growing in the food. Two common toxin-producing bacteria are B. cereus, and S. aureus. What foods are likely to cause poisoning by these bacteria. What precautions in food handling could prevent this type of poisoning (Here, especially consider temp maintenance).**

S. aureus- meat and meat products, poultry and egg products, salads (macaroni, potato, tuna, egg, etc.), cream-filled desserts, sandwhich fillings (?), milk and dairy

prevention: wash hands, don’t prepare if you have a nose or eye infection, wounds or

skin infections on hands and wrists, keep prep area clean, keep hot foods >140 F and cold foods <40 F, store cooked food in a wide, shallow container

B. cereus- meat, milk, veg, fish (diarrheal\*), rice (vomiting\*), potato, pasta, cheese, sauces, pudding, soup, casserole, pastries, and salads

\* apparently there are 2 forms of B. cereus poisoning- one causes dairrhea, and the

other causes vomiting

prevention: keep hot food >140 F, steam and pressure cooking, roasting, frying, and grilling kills spores, heat 5 min at 133 F to remove diarrheal toxin or 259 F for 90 min to remove emetic toxin (the one that causes vomiting)

6. You can determine whether Shiga-like toxins or toxins associated with *Clostridium difficile* are present in feces obtained from a person with diarrhea using antibody neutralization assays with appropriate controls by adding the patients sample to wells with cell tissue cultures and then adding serum from an animal immunized for each of the toxins to specified wells. If the cells die in the non serum wells and survive in the sample+serum wells, the patient has the toxin present. If they die in both sample well and sample+ serum well, the patient does not have that toxin present.

**7. Listeriosis**

Vegetables, meats, and other foods you eat can get infected with the bacteria if they come in contact with contaminated soil or manure. *Listeria monocytogenes* is Gram positive and is found in soil and water. Animals can carry the bacterium without appearing ill and can contaminate foods of animal origin, such as meats and dairy products. When *Listeria* bacteria get into a food processing factory, they can live there for years, sometimes contaminating food products. The bacterium has been found in a variety of raw foods, such as uncooked meats and vegetables, as well as in foods that become contaminated after cooking or processing, such as soft cheeses, processed meats such as hot dogs and deli meat (both products in factory-sealed packages and products sold at deli counters), and smoked seafood. Unpasteurized (raw) milk and cheeses and other foods made from unpasteurized milk are particularly likely to contain the bacterium.

If you eat the contaminated products, you may get sick. Pregnant women, developing fetuses, newborns, and adults with weakened immune systems are at increased risk. The bacteria most often cause a gastrointestinal illness. In some cases, you can develop a blood infection (septicemia) or inflammation of the covering of the brain (meningitis).

Infection in early pregnancy generally leads to miscarriage. The bacteria may cross the placenta and infect the developing baby.

In infants, symptoms of listeriosis may be seen in the first few days of life and may include:

· Loss of appetite

· Lethargy

· Jaundice

· Vomiting

· Respiratory distress (usually pneumonia)

· Shock

· Skin rash

· Increased pressure inside the skull (due to meningitis) possibly causing suture separation

Late-appearing infection in the infant and infection in children is often seen as meningitis.

Infants who survive listeriosis may have long-term neurological damage and delayed development.

In adults, the disease may take many forms depending on what organ or organ systems are infected. It may occur as meningitis, pneumonia, septicemia, and endocarditis, or in milder form as abscesses, skin lesion, and conjunctivitis.

Diagnosis is obtained by blood or CSF culture. Treatment with antibiotics.